Ectomycorrhizal fungi of *Salix rotundifolia* III. Resynthesized mycorrhizal complexes and their surface phosphatase activities

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Pure culture isolates were obtained from fungi fruiting in the vicinity of dwarf willows at Barrow and Cape Simpson, Alaska. Four of these isolates and one isolate from Maryland were tested for their ability to form ectomycorrhizae with cuttings of *Salix rotundifolia* under controlled environmental conditions. Isolates of *Entoloma sericeum*, *Hebeloma pusillum*, and *Cenococcum geophilum* from Barrow and Cape Simpson, Alaska all formed typical ectomycorrhizae with *S. rotundifolia*, while an isolate of *C. geophilum* from a temperate ecosystem (Maryland) did not.

All of the ectomycorrhizae synthesized with *S. rotundifolia*, plus uncolonized roots, demonstrated an ability to hydrolyze *p*-nitrophenyl phosphate at a pH of 4.7. The acid phosphatase activity of *E. sericeum* ectomycorrhizae was from 10 to 40 times as great as that demonstrated by other mycorrhizal and nonmycorrhizal roots on a surface area basis.

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Des isolats en culture pure ont été obtenus à partir de fructifications de champignons croissant à proximité de saules nains à Barrow et Cape Simpson, Alaska. Pour quatre de ces isolats et un isolat provenant du Maryland, on a évalué l'aptitude à former des ectomycorhizes avec des boutures de *Salix rotundifolia* dans des conditions environnementales contrôlées. Les isolats d'*Entoloma sericeum*, *Hebeloma pusillum* et *Cenococcum geophilum* provenant de Barrow et Cape Simpson peuvent former des ectomycorhizes typiques avec *S. rotundifolia*, tandis qu'un isolat de *C. geophilum* provenant d'un écosystème tempéré (Maryland) en est incapable.

Toutes les ectomycorhizes synthétisées avec *S. rotundifolia* ainsi que les racines non colonisées sont capables d'hydrolyser le *p*-nitrophényl phosphate à un pH de 4,7. Pour une surface donnée, l'activité phosphatasique acide des ectomycorhizes d'*E. sericeum* est de 10 à 40 fois plus grande que celle des autres racines mycorhizées et non mycorhizées.

[Traduit par le journal]

Introduction

The presence of ectomycorrhizae on the roots of the dwarf deciduous shrub *Salix rotundifolia* Trautv. was first noted for specimens collected from Novya Sembya by Hesselman (1900). Hesselman's findings were later confirmed for plants from arctic tundra of the U.S.S.R. (Katenin 1964) and Alaska (Antibus and Linkins 1978; Miller and Laursen 1978). Although the occurrence of ectomycorrhizae in *S. rotundifolia* was noted early, very little research has been directed at finding out which fungi are responsible for these observed associations. A list of probable ectomycorrhizal associates of various *Salix* species was given by Trappe (1962). Included in Trappe's list were several willow species which occur as dwarfed forms in alpine and arctic tundra. However, no mention was made of *S. rotundifolia*.

Although several techniques have been employed to demonstrate the direct connection between a suspected mycobiont and its host plant, the most commonly accepted procedure involves the successful synthesis of mycorrhizae between mycobionts and host plant grown under axenic conditions (Zak 1973). Few such studies have been conducted using *Salix* species; however, Fontana (1963) reported the synthesis of ectomycorrhizae between *S. purpurea* L. and *Hebeloma hiemale* Bres. Attempts to induce mycorrhizal formation between suspected willow mycobionts and alternate hosts, such as *Pinus virginiana* Mill., have met with limited success (Hacskaylo and Bruchet 1972). Recently Gaie (1977) developed a promising technique for synthesizing ectomycorrhizae utilizing surface sterilized woody cuttings of *S. repens* L. Using this technique Gaie successfully synthesized ectomycorrhizae between *S. repens* and both *Paxillus involutus* (Batsch) Fr. and *Pisolithus arhizus* (Pers.) Rausch.

Richards and Wilson (1963) stated that an understanding of why certain types of ectomycorrhizae develop under various conditions can come about only when we understand the functional aspects of these structures. One function frequently ascribed to ectomycorrhizae is

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their ability to increase solubilization of insoluble forms of soil organic phosphate (Bowen 1973). Surface acid phosphomonoesterase activity has been used as a measure of this mycorrhizal function in both ectomycorrhizae (Bartlett and Lewis 1973) and ectomycorrhizal fungi (Ho and Zak 1979; Calleja et al. 1980). It has been suggested by Ho and Zak (1979) that a relationship may exist between the acid phosphatase activities of ectomycorrhizal fungi, and their relative effectiveness as mycobionts.

The purpose of the present investigation was to obtain pure cultures of fungi found fruiting in the vicinity of dwarf willows at Barrow, Alaska, and to determine which of these isolates were capable of forming ectomycorrhizae with *S. rotundifolia* under controlled environmental conditions. In addition, the effect of different host–mycobiont combinations on the surface acid phosphatase activities of resynthesized ectomycorrhizae was examined.

Materials and methods

Fungal isolation procedures

Attempts were made to isolate suspected mycorrhizal fungi at Barrow and Cape Simpson, Alaska, during the summers of 1976 and 1977. The procedures employed included culturing tissues from fungal fruiting bodies found in the vicinity of *Salix* species, germinating spores discharged by these fruiting bodies, and obtaining mycelial growth from surface sterilized *S. rotundifolia* mycorrhizal roots.

The procedures utilized to obtain cultures from fruiting bodies and spores were as described by Palmer (1971). Two solid media, a modified Hagem's medium, and a yeast peptone glucose medium (Antibus 1980), were used in all isolation attempts.

Isolations were made from *S. rotundifolia* mycorrhizae observed to be very common in root tip counts using a modification of the method of Zak and Bryan (1963). Mycorrhizae were cut into 1-cm lengths and washed in a 1% solution of Tween 20 for 2 min. Following a wash in sterile distilled water, mycorrhizae were placed in 1.5% H_2O_2 for 2 min, 20% Chlorox for 2 min, and were then washed three times in sterile distilled water. Four segments were placed in each petri dish and replicates incubated at 5 and 20°C. Cultures were checked weekly for signs of growth; contaminated plates were immediately discarded.

Starter plants

Cuttings of *S. rotundifolia* plants used in resynthesis experiments were obtained from blocks of tundra shipped by air from Barrow, Alaska, in July 1978. The blocks, approximately 30 cm square and 15 cm deep, were maintained for 1 month at 20°C and watered twice weekly with distilled water.

Each stem cutting consisted of one leaf with attendant axillary bud and a section of primary stem from approximately 5 mm below the leaf node to 5 mm below the next leaf node. Cuttings were immediately placed in sterile distilled water in plastic petri dishes to prevent drying. The cuttings were surface sterilized in 1.5% H_2O_2 for 30 s, followed by three washes in sterile water. The basal ends were dipped in a slurry of sterile water and Rootone F (Amchem Products Inc.) prior to planting in pyrex dishes with dimensions of 22 × 11 × 7 cm. The rooting medium consisted of a mixture of sieved peat moss and vermiculite (1:20 v/v) as described by Marx and Zak (1965). Each pyrex dish received 500 mL of the peat–vermiculite mixture and 300 mL of ½-strength Hoagland's solution (Hoagland and Arnon 1938). The dishes were then sealed in transparent autoclavable bags and autoclaved for 30 min. After cooling, 30 cuttings were planted in each dish, the autoclavable bag was resealed, and the dishes placed in a lighted growth chamber at 20°C.

Fungal starters

Fungal isolates utilized in the resynthesis studies with *S. rotundifolia* were as follows: *Cenococcum geophilum* Fr. (VT 715, isolated from *Pinus virginiana* roots from Maryland by J. F. Worley), *C. geophilum* (*VT 1005*, isolated from *S. rotundifolia* mycorrhiza at Cape Simpson, Alaska), *C. geophilum* (VT 1004, isolated from soil at Barrow, Alaska), *Hebeloma pusillum* (VT 1002, isolated from a fruiting body at Barrow, Alaska), and *Entoloma sericeum* (VT 1003, isolated from a fruiting body at Barrow, Alaska).

Starters of *H. pusillum* were grown in petri dishes containing 15 mL of solid yeast peptone glucose medium, while all other isolates were grown on a modified Hagem's medium. Once mycelial growth had developed on agar plates, two 4-mm-diameter plugs of each isolate were transferred to 125-mL flasks containing 25 mL of the appropriate liquid medium. Inoculated flasks were placed in a growth chamber at 20°C for 28 days.

Inoculation and care of plants

Resynthesis medium consisted of the peat–vermiculite mixture (1:20 v/v), 100 mL per 250-mL flask. Each flask received 50 mL of ½-strength Hoagland's solution amended with 27.8 m*M* D-glucose, 2.96 μm thiamin–HCl, and 0.02 μ*M* biotin. The pH of the medium 24 h after autoclaving varied between 5.6 and 5.8.

After 28 days of growth, four fungal starter plugs of each isolate were transferred to 25 mL of sterile medium in a sterile Eberbach semimicro-homogenizer. The mycelium was dispersed by two 10-s bursts at full speed on a Waring blender and 2 mL of this suspension was placed into each 250-mL flask containing the sterile peat–vermiculite.

After the fungal inoculum had grown for 21 days, cuttings for resynthesis were selected on the basis of uniform size, adventitious root formation, and overall vigor. These cuttings were subjected again to the previously described sterilization regime, and aseptically transferred, one cutting per flask. Fifteen replicate flasks per isolate plus control flasks (no fungus) were established at this time.

All flasks were placed in a lighted growth chamber at 20°C receiving 18 h of light and 6 h of dark. Light intensity was measured at the level of the medium once a month with an ISCO SR recording spectroradiometer. During the 18-h photoperiod plants received 12 h of fluorescent plus incandescent light and 6 h of fluorescent light only. The photosynthetically active radiation during these two periods averaged 0.223 and 0.186 cal cm^{-2} min^{-1} (1 cal = 4.184 J), respectively.

During the study period plants were checked weekly for visible signs of contamination with contaminated flasks being discarded. After 90 days, each flask received 10 mL of sterile ½-strength Hoagland's solution.

Root structure

After 180 days, all surviving plants were harvested. Root systems were washed free of adhering peat–vermiculite mixture under cold running water. Entire root systems and individual short roots were fixed for light microscopy in chromic acid–acidic acid–formalin (CRAF III) fixative (Johansen 1940) at 2°C overnight. Roots were then dehydrated in an ethanol–TBA series and embedded in paraffin. Sections cut at 5–10 μm were stained with safranin and fast green (Johansen 1940). Samples of mycorrhizae and control roots for scanning electron microscope (SEM) examination were fixed, postfixed, and critical-point dried according to the procedure of Kinden and Brown (1975). Samples were coated with gold–palladium and examined with an AMR-900 scanning electron microscope.

Acid phosphatase assay

Surface acid phosphomonesterase (EC 3.1.3.2) activities of experimental roots were measured using a modification of the *p*-nitrophenyl phosphate (PNPP) technique of Bartlett and Lewis (1973). Each experimental vial contained six unbranched root tips of each mycorrhizal root type or control roots in 0.5 mL of 1 m*M* sodium citrate buffer, pH 4.7 (the pH optima of beech mycorrhizae, Bartlett and Lewis (1973)) plus 0.5 mL of 20 m*M* *p*-nitrophenyl phosphate in the same buffer adjusted to pH 5.5. Vials were incubated on a shaker at 25°C for 2 or 3 h; controls contained buffer and substrate without roots. At the end of the incubation period, roots were removed for surface area determinations from length and width measurements. Upon removal of roots each vial received 4 mL of 0.1 *N* NaOH and absorbance was read at 410 nm on a Gilford model 250 spectrophotometer. The amount of substrate hydrolyzed was then determined using a standard curve obtained with an alkaline solution of *p*-nitrophenol. Activities proved to be linear over the 3-h incubation period, in addition no absorption of *p*-nitrophenol was observed to occur over this time period. Activity estimates based on surface area were analyzed by the Newman-Keuls multiple range test as outlined by Zar (1974).

Results and discussion

Resynthesis experiments

A list of fungi found fruiting in the vicinity of dwarf willows during the summers of 1976 and 1977 is given in Table 1. Attempts to form mycorrhizae with *S. rotundifolia* were made only with those fungi which demonstrated substantial growth in pure culture, that is, *H. pusillum*, *E. sericeum*, and *C. geophilum* (isolates from Barrow, Cape Simpson, and Maryland). The results are given below.

S. rotundifolia *plus* H. pusillum

Mycorrhizae simple to profusely branched, individual elements cylindric 1–5(10) mm × 0.1–0.2 mm, white to yellowish-white (Fig. 1). Mantle generally tomentose, but appearing nearly smooth in some SEM preparations (Figs. 1, 2), emergent hyphae 2–5 μm in diameter, hyphal cells 10–20 μm long, clamp connections at every third or fourth cell, intervening cells with clampless septa. Mantle ranging in thickness from 2–19 μm with a mean thickness of 9 μm, consisting of interwoven to subparallel hyphae (Figs. 3, 4, 5). A well-developed Hartig net of mycelium 2–3 μm thick penetrates between the cells of one or two cortical layers (Figs. 4, 5). No intracellular invasion observed.

TABLE 1. List of fungal species from which attempts were made to obtain cultural isolates. All species were collected in the vicinity of dwarf willows at Barrow or Cape Simpson, Alaska

Ascomycetes	
++	*Cenococcum geophilum* Fr.
−	*Helvella corium* (Weberb.) Massie
Basidiomycetes	
−	*Cortinarius cinereoviolaceus* (Fr.) J. Lange
+	*C. mucosus* (Bull. ex Fr.) Kickx.
−	*C. huronensis* var. *huronensis* Ammirati
++	*Entoloma sericeum* (Bull. ex Merat) Quelet.
+	*Hebeloma sp.*
++	*Hebeloma pusillum* J. Lange
−	*Laccaria laccata* (Fr.) Berk. and Br.
−	*L. striatula* (Peck) Pk.
+	*Lactarius lanceolatus* O. K. Miller and G. A. Laursen
−	*Russula emetic* A. Schaeff. ex. Fr. var. *alpestris* Bond.
−	*R. xerampelina* Schaeff. ex. Fr. var. *pascua* Moller and J. Schaeff.

NOTE: −, no growth; +, limited growth; ++, substantial growth.

Laursen (1975) felt that *H. pusillum* was ectomycorrhizal with dwarf willows at Barrow; however, this contention was based only upon field observations. Ohenoja (1971) felt that some species of *Hebeloma*, including *H. pusillum*, might not be obligatorily mycorrhizal as she observed fruiting bodies on bare soil far removed from willow plants. Hacskaylo and Bruchet (1972) demonstrated that a majority of the 26 species of *Hebeloma* they tested were capable of forming ectomycorrhizae with *P. virginiana*. Their isolate of *H. pusillum* (collected near *Salix aurita*) did not form ectomycorrhizae with *P. virginiana*, leading them to conclude that this fungus was either nonmycorrhizal or was a specific associate of willows. The association between *H. pusillum* and *S. rotundifolia* synthesized in the present study can be classified as a typical ectomycorrhizal association (Zak 1973) and confirms the ability of this fungus to form ectomycorrhizae. Ectomycorrhizae synthesized between *H. hiemale* and *S. purpurea* have been described in detail by Fontana (1963), and between *H. crustuliniforme* (Scop, ex Fr.) Bk. & Br. and *Pseudotsuga menziesii* (Mirb) Franco. by Trappe (1967). *Salix rotundifolia* plus *H. pusillum* mycorrhizae resemble these previously described associations in

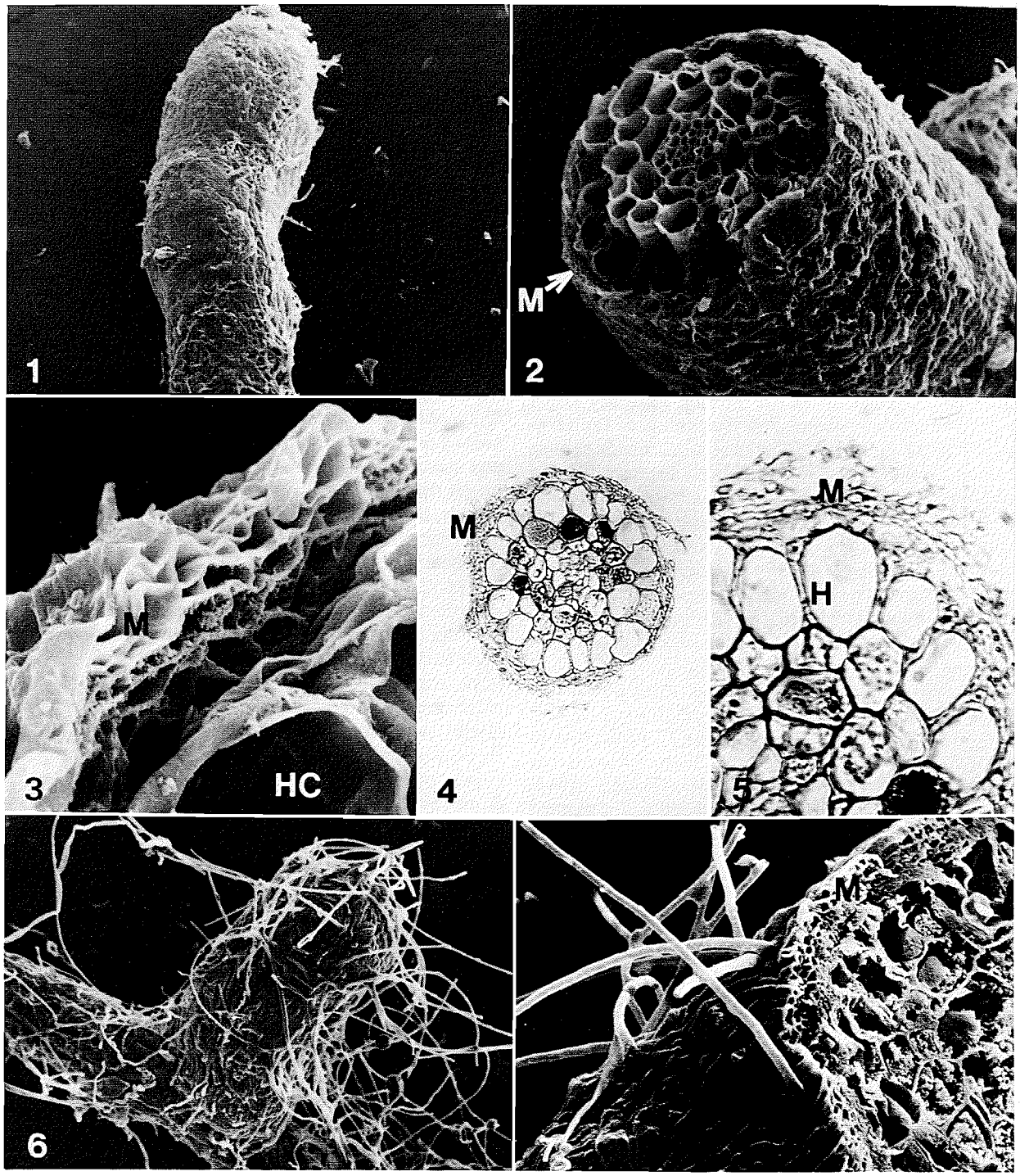


FIG. 1. SEM preparation of *S. rotundifolia* plus *H. pusillum* mycorrhiza. ×150. FIG. 2. SEM of the same mycorrhizal complex showing mantle (M) features. ×350. FIG. 3. SEM showing details of mantle structure and host cell (HC) for *S. rotundifolia* plus *H. pusillum* mycorrhiza. ×3400. FIG. 4. Cross section of *S. rotundifolia* plus *H. pusillum* mycorrhiza. ×200. FIG. 5. Cross section of the same mycorrhizal complex showing mantle and Hartig net (H). ×600. FIG. 6. SEM preparation of *S. rotundifolia* plus *C. geophilum* mycorrhiza synthesized in pure culture. ×150. FIG. 7. SEM of the same mycorrhizal complex showing mantle in detail ×450.

mantle color, mantle surface features, and hyphal diameters. *Hebeloma* mycorrhizae formed in the present study have mantles which on the average are thinner than previously described *Hebeloma* mycorrhizae (Trappe 1967). A great deal of variability was observed to exist in the surface features of the mantle and the thickness of the mantle formed by *H. pusillum*. It should also be noted that clamp connections are found at only every third or fourth septum in the mycelium of *H. pusillum* mycorrhizae, not at every septum as seen in other *Hebeloma* mycorrhizae (Trappe 1967).

S. rotundifolia *plus* C. geophilum

Mycorrhizae simple to sparsely branched, individual elements stalked, cylindric to subglobose 0.5–1(3) mm × 0.15–0.30 mm, black (Fig. 6). Mantle smooth to tomentose, emergent hyphae brownish-black 3–6 μm in thickness, hyphae on mantle surface generally larger and more strongly pigmented than those of the inner mantle (Figs. 8, 9). In tangential section, mantle hyphae exhibit a stellate arrangement. A well-developed Hartig net of nearly hyaline mycelium 1–3 μm in diameter penetrates between the cells of the outer one or two cortical layers (Figs. 8, 9). No intracellular penetration observed. Ectomycorrhizae formed by *C. geophilum* isolates from Barrow and Cape Simpson were identical in all morphological and anatomical aspects. The Maryland isolate failed to form ectomycorrhizae with *S. rotundifolia* in three separate experiments.

Cenococcum geophilum is known to be capable of forming ectomycorrhizae with a wide variety of host plants (Trappe 1964). The presence of *C. geophilum* on the roots of several dwarf alpine willows in Europe was reported by Dominik et al. (1954). Trappe (1964) reported *C. geophilum* to be mycorrhizal with a number of *Salix* species, including an unidentified species from tundra near Kotzebue Sound, Alaska. Isolates of *C. geophilum* from Barrow and Cape Simpson formed ectomycorrhizae which were identical in coloration, morphology, and mantle structure to those described by Hatch (1934). The Maryland isolate repeatedly failed to form ectomycorrhizae although prolific growth was noted on the surface of and in the vicinity of *S. rotundifolia* roots. It seems likely that this isolate has either lost its ability to form ectomycorrhizae through years of subculturing, or that it is incapable of penetrating the roots of *S. rotundifolia*.

S. rotundifolia *plus* E. sericeum

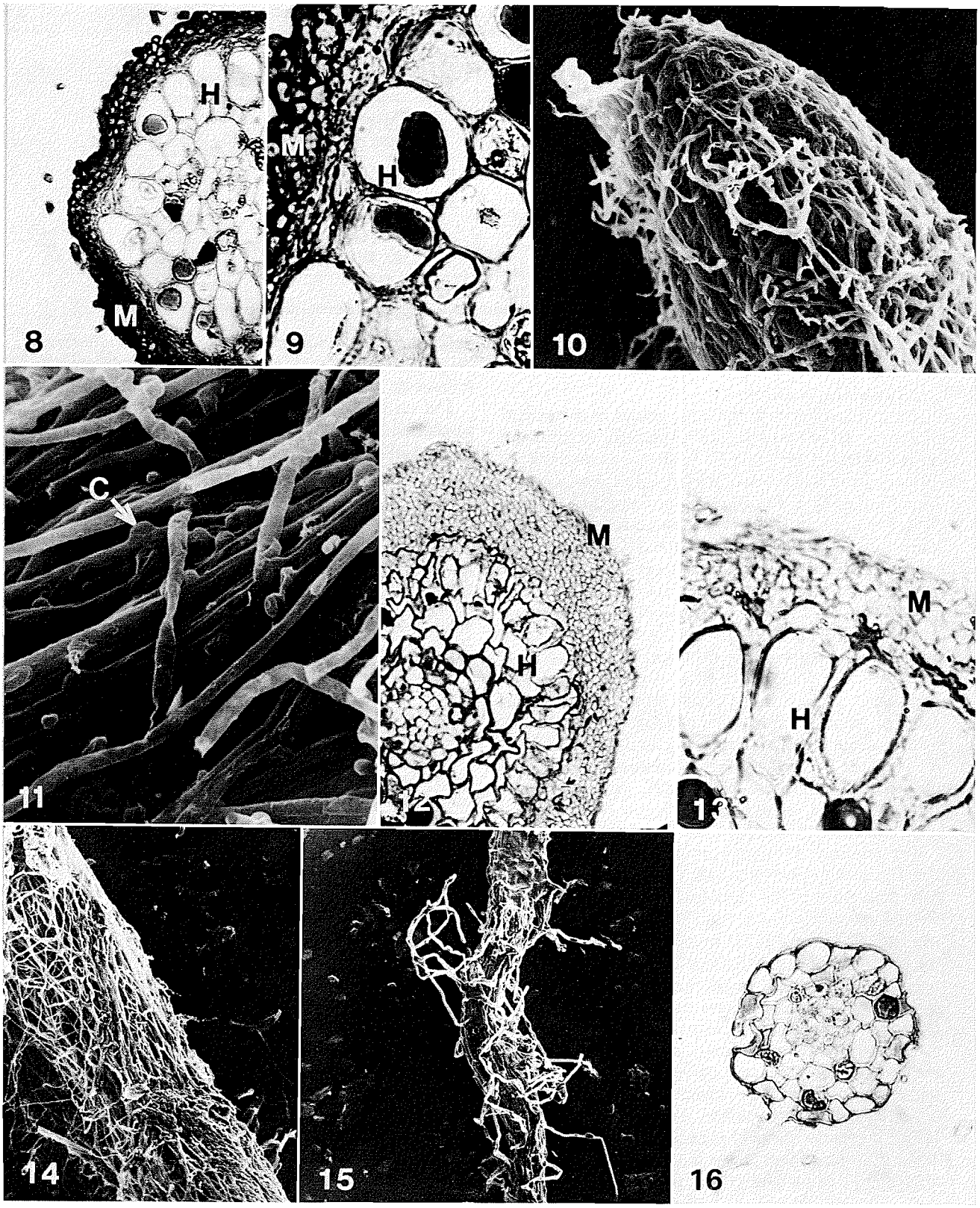
Mycorrhizae simple to profusely branched, individual elements occasionally stalked, cylindric 5–10(25) mm × 0.15–0.30, white to yellowish-white. Mantle smooth to short tomentose, often with appressed felty layer. SEM preparations demonstrate a dense covering of loosely attached mycelium (Figs. 10, 14), attached mycelium 2.5–4 μm in diameter with clamp connections at nearly all septa. Mantle ranging from 6–58 μm with a mean thickness of 22 μm, consisting of parallel hyphal cells with abundant clamp connections, the hyphae arranged parallel to the long axis of the root (Figs. 11, 12, 13). Hartig net generally poorly developed of mycelium 2.5–5 μm thick, penetrating between the cells of one or two cortical layers (Figs. 12, 13).

Species of *Entoloma* (*Rhodophyllus* of some authors) have been observed fruiting in the vicinity of dwarf willows by numerous authors (Favre 1955; Lange 1957; Kobayasi et al. 1971). Kobayasi et al. (1971) stated that *Rhodophyllus sericeus* (Bull.) Quel. (= *E. sericeum*) formed mycorrhizae with *S. herbacea*. This statement was apparently based on observations of the habitat of fungal fruiting. Ohenoja (1971) felt that *Rhodophyllus* species, like *Hebeloma* species might not be obligatorily mycorrhizal. Overall, few investigations have been conducted into the ecological relationships of *Entoloma* species (Horak 1980), and although some groups are suspected to be mycorrhizae formers, most are thought to be decomposers. To date very few attempts have been made to synthesize mycorrhizae between suspected host plants and members of the Entolomataceae. Using oak plants grown in sterilized soil, Zerova and Rozhenko (1966) were able to form ectomycorrhizae with *E. erophilum* (Fr.) Karst. and *E. sericeum* but not with *E. cylpeatum* (Fr.) Kummer. Mycorrhizae formed between *S. rotundifolia* and *E. sericeum* in the present study demonstrated the characteristics of typical ectomycorrhizae. These mycorrhizae superficially resembled those formed by *H. pusillum*, but clearly differed from the latter in thickness of mantle, structure of the mantle, and abundance of clamp connections in mantle hyphae. Present results are also significant in that they represent the first report of synthesis of mycorrhizae with *Entoloma* in a closed axenic system.

S. rotundifolia *controls*

Root systems highly branched, individual roots cylindric, ultimate roots 0.5–2(4) cm × 0.05–0.1 mm, white to yellowish-white (Fig. 15). Ultimate roots with abundant root hairs 6–13 μm in diameter (Figs. 15, 16). No signs of fungal growth or mycorrhizal formation

FIG. 8. Cross section of *S. rotundifolia* plus *C. geophilum* mycorrhiza. ×400. FIG. 9. Cross section showing details of mantle and Hartig net of the same mycorrhizal complex. ×1000. FIG. 10. SEM preparation of *S. rotundifolia* plus *E. sericeum* mycorrhiza. ×350. FIG. 11. SEM preparation of the same mycorrhizal complex showing details of mantle and clamp connections (C). ×1500. FIG. 12. Cross section of same mycorrhizal complex. ×400. FIG. 13. Cross section of same mycorrhizal complex showing details of mantle and Hartig net. ×1200. FIG. 14. SEM of *S. rotundifolia* plus *E. sericeum* mycorrhiza showing attached fungal mycelium. ×150. FIG. 15. SEM preparation of an uncolonized *S. rotundifolia* root. ×75. FIG. 16. Cross section of the same. ×450.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16

TABLE 2. The results of *p*-nitrophenyl phosphatase assays conducted on roots from two separate resynthesis experiments

Complex	Experiment I		Experiment II	
	n	PNPP hydrolyzed (ng mm^{-2} h^{-1})	*n*	PNPP hydrolyzed (ng mm^{-2} h^{-1})
S. rotundifolia uncolonized	3	50±21*a**†	6	90±11*a**†
+ *Hebeloma pusillum*	5	86±17*a*	6	77±22*a*
+ *Cenococcum geophilum* from Cape Simpson	6	110±13*a*	6	172±25*b*
+ *Cenococcum geophilum* from Barrow	3	92±14*a*	—	—
+ *Entoloma sericeum*	7	2139±538*b*	4	1760±150*c*

*Mean ± standard deviation.
†Numbers followed by the same letter within each experiment are not significantly different at the 5% probability level.

were observed on any of the root systems of control plants.

Phosphatase activities

The results of acid phosphatase assays on *S. rotundifolia* roots from two separate resynthesis experiments are summarized in Table 2. In both experiments the greatest rate of hydrolysis of *p*-nitrophenyl phosphate was demonstrated by *E. sericeum* mycorrhizae. In both cases the acid phosphatase activities of these ectomycorrhizae were significantly greater than those shown by other synthesized mycorrhizae and uncolonized (control) roots. These results indicated that colonization by *E. sericeum* resulted in a 20- to 40-fold increase in acid phosphatase activity of *S. rotundifolia* roots. In both experiments ectomycorrhizae formed by the Cape Simpson isolate of *C. geophilum* demonstrated the second highest acid phosphatase activity. In the second set of experiments the mean phosphatase activity of ectomycorrhizae formed by this isolate was significantly higher than that of either *H. pusillum* mycorrhizae or uncolonized roots. Acid phosphatase activity estimates for *H. pusillum* mycorrhizae were not significantly different from those of uncolonized roots in either experiment.

The presence of surface *p*-nitrophenyl phosphatase activity in field-collected beech ectomycorrhizae was first demonstrated by Woolhouse (1969) and later studied in beech by Bartlett and Lewis (1973). Williamson and Alexander (1975) found that histochemically, the acid phosphatase activity of beech ectomycorrhizae was restricted to the fungal mantle. In addition they showed that acid phosphatase activity of beech mycorrhizae was up to eight times as great as that of uninfected roots. The identity of the fungal symbiont or symbionts responsible for this acid phosphatase activity was unknown in these experiments dealing with field-collected beech mycorrhizae. Recently, Ho and Zak (1979) compared the acid phosphatase activities of six ectomycorrhizal fungi grown in sterile liquid culture. A large degree of variability was found to exist between the acid *p*-nitrophenyl phosphatase activities of the various isolates, with the activity of *H. crustuliniforme* being some 35 times as great as that exhibited by *Piloderma bicolor* (Peck) Julich.

The results of the present study using mycorrhizae synthesized under controlled conditions confirm the findings of Ho and Zak (1979), that differences do exist in the abilities of different ectomycorrhizal fungi to hydrolyze *p*-nitrophenyl phosphate. Colonization of roots by *H. pusillum* did not appear to enhance the acid phosphatase activity of *S. rotundifolia* roots under the present experimental conditions. Colonization by *C. geophilum* enhanced phosphatase activity only slightly, while colonization by *E. sericeum* resulted in a very distinct increase in phosphatase activity. One possible explanation for these observed differences can be found in the anatomy and morphology of these ectomycorrhizae. *Hebeloma pusillum* mycorrhizae, which expressed the lowest phosphatase activities, formed a very thin mantle, which at times covered only part of the root surface. In some SEM preparations the mantle appeared nearly smooth. *Cenococcum geophilum* mycorrhizae, which formed a thicker mantle than *H. pusillum* and had a greater amount of attached mycelium, demonstrated a slightly increased phosphatase activity. Mycorrhizae of *E. sericeum* formed the thickest mantle and demonstrated the greatest amount of attached mycelium. The surface area of this attached mycelium was not taken into account in measurements of root surface area, and therefore probably resulted in an overestimate of the phosphatase activity per unit surface area of those mycorrhizae. It is also possible that the pH at which the assay was conducted was more favorable to *E. sericeum* than to the other mycorrhizae examined in the present study.

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